Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes (hiPSC-CMs) as a Platform for Modeling Arrhythmias

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Lisa Lin, Tiffany Barszczewski, Patrick G. Burgon, and Glen F. Tibbits

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Lisa Lin and Tiffany Barszczewski contributed equally with all other contributors.

L. Lin
Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada
e-mail: lla5@sfu.ca

T. Barszczewski · G. F. Tibbits (✉)
Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada

Cellular and Regenerative Medicine Centre, BC Children's Hospital Research Institute, Vancouver, BC, Canada
e-mail: tiffany_barszczewski@sfu.ca; tibbits@sfu.ca

P. G. Burgon (✉)
Department of Chemistry and Earth Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar
e-mail: patrick.burgon@qu.edu.qa

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Abstract

Cardiac arrhythmias can arise due to a host of both genetic and acquired factors. Specifically, the genetic basis of arrhythmogenesis is not fully understood due to the lack of robust models that reliably recapitulate human physiology. Human-induced pluripotent stem cells (hiPSCs) have strengthened regenerative medicine by producing cells that bear the genetic signature of patients being studied. Upon differentiation into hiPSC-derived cardiomyocytes (hiPSC-CMs), these cells can be used to phenotype known mutations or suspected variants that may contribute to abnormal electrical activity in the heart. Furthermore, novel therapeutics can be screened for the management and treatment of arrhythmias in patient-specific hiPSC-CMs. In this chapter, we will briefly discuss the practical utility of hiPSC-CMs to study inherited arrhythmias with a specific focus on atrial fibrillation (AF), catecholaminergic polymorphic ventricular tachycardia (CPVT), and disruptive electrical events that may occur in patients with hypertrophic cardiomyopathy (HCM). We will describe an investigative pipeline that integrates genome editing, tissue engineering, biobanking, and systems biology as complementary approaches. Together, these various applications are directed toward a common goal of bench-to-bedside characterization of arrhythmias in patient-specific hiPSC-CMs.

Keywords

Arrhythmia · Cardiomyocytes · Cardiomyopathy · Fibrillation · hiPSCs · Pluripotent

Abbreviations

AF	Atrial fibrillation
AFM	Atomic force microscopy
CiPA	Comprehensive in vitro proarrhythmia assay
DCM	Dilated cardiomyopathy
ddPCR	Droplet digital polymerase chain reaction
ER	Endoplasmic reticulum
FFA	Free fatty acids
FFT	Fast Fourier transform
GECI	Genetically encoded calcium indicator
GEVI	Genetically encoded voltage indicator
GWAS	Genome-wide association studies
HCM	Hypertrophic cardiomyopathy
hESCs	Human embryonic stem cells
hiPSC-CMs	Human-induced pluripotent stem cell-derived cardiomyocytes

hiPSCs	Human-induced pluripotent stem cells
LQTS	Long QT syndrome
LTCC	L-type calcium channel
MEA	Multielectrode array
M-MLV	Modified Moloney leukemia virus
MS	Mass spectrometry
PBMCs	Peripheral blood mononuclear cells
pegRNA	Prime editing guide RNA
PTM	Posttranslational modification
QC	Quality control
qPCR	Quantitative polymerase chain reaction
RyR	Ryanodine receptor
SCD	Sudden cardiac death
SNP	Single nucleotide polymorphism
SR	Sarcoplasmic reticulum
TEM	Transmission electron microscopy
TTs	T-tubules
VF	Ventricular fibrillation

1 Introduction

Human-induced pluripotent stem cells (hiPSCs) continue to enhance the study of complex diseases – especially those that contribute to certain cardiac pathophysiology – by putting personalized medicine within arm's reach. With this progress, researchers now have unprecedented access to cell-type and patient-specific assays, including high-throughput drug screening and disease modeling. Shinya Yamanaka was awarded the 2012 Nobel Prize in Physiology or Medicine for his team's transformative work in 2007, generating pluripotent stem cells from reprogrammed somatic cells (Takahashi et al. 2007). The hiPSCs share similar pluripotency and differentiation capacities to human embryonic stem cells (hESCs). The initial use of somatic cells circumvents the ethical challenges of using hESCs and are easily obtained through minimally invasive procedures (e.g., skin punch biopsies, blood draws, and buccal swabs). hiPSC lines can be maintained in culture endlessly and cryopreserved for long-term study.

Since Yamanaka's seminal publication, tremendous effort has been made to facilitate the differentiation of hESCs and hiPSCs into functional cardiomyocytes (Burridge et al. 2014; Lian et al. 2013). The process employs a chemically defined small molecule protocol to initially direct the hiPSCs toward a mesodermal lineage by adding a glycogen synthase kinase-3 (GSK-3) inhibitor. The subsequent cardiac specification is induced by a Wnt pathway inhibitor to produce spontaneously beating, primarily ventricular, hiPSC-CMs. Other cardiac cell types, namely, atrial and nodal hiPSC-CMs, can also be specified by the temporal addition of retinoic acid during the differentiation process (Protze et al. 2017).

With current advancements in bench-to-bedside research, hiPSCs from patients harboring pathogenic mutants (or suspect variants identified by genome-wide

association studies (GWAS)) can be generated and differentiated into the hiPSC-CMs to model heart rhythm conditions (Cagavi et al. 2018). To put the importance of this research field into perspective, an estimated three million individuals in the United States are affected by atrial fibrillation (AF). With a rapidly aging population, the number is expected to rise to upwards of 12 million by 2030 (Colilla et al. 2013; Miyasaka et al. 2006). In parallel, ventricular arrhythmias lapse into high incidences of hospitalization and may be responsible for up to 400,000 sudden cardiac death (SCD) cases each year (Khurshid et al. 2018; Roberts-Thomson et al. 2011). Inherited arrhythmias are a subset of these electrical events and are arguably more sinister in nature; by which an individual may experience ventricular tachycardia once in their life due to physical trauma or a myocardial infarct, patients who are genetically predisposed to an arrhythmic condition may face obstacles operating against their health daily. This predisposition can dramatically decrease the quality of life for individuals who may also be required to compromise activities such as exercise and play for extra caution.

The genetic and mechanistic bases of inherited rhythmic disorders are complicated, to say the least, although strategies to link gene variants to cardiac disease-associated arrhythmias have been ongoing for more than 30 years. Thus, an interdisciplinary approach (descendant from systems biology) using hiPSC-CMs in vitro is required to derive a mechanistic explanation for a given patient's arrhythmogenic phenotype in vivo. Furthermore, when a variant is identified in the patient's genome, a comprehensive characterization of that variant should be carried out.

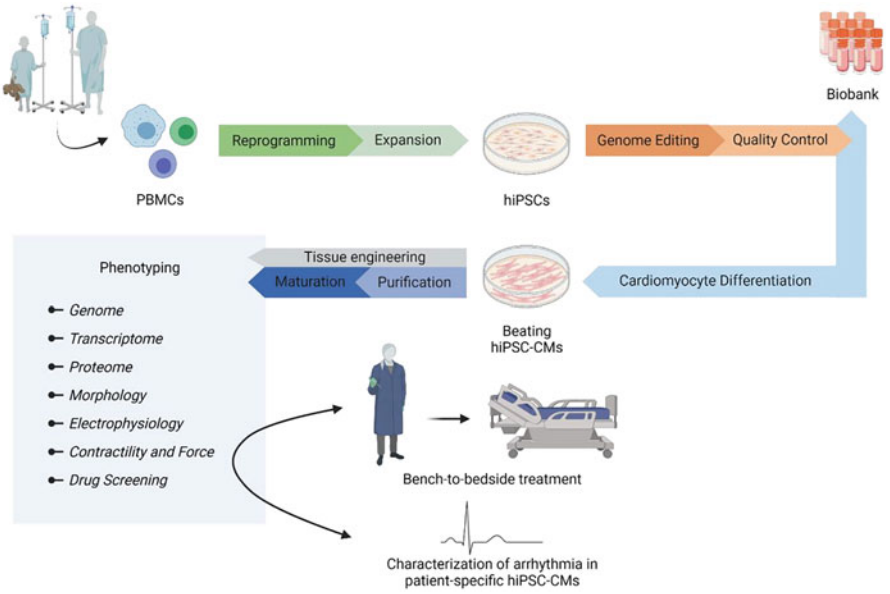

Fig. 1 Pipeline for the use of hiPSC-CM. *Genome edited hiPSCs that have undergone Q/C tests

In this chapter, we briefly describe a pipeline that consolidates hiPSC generation from patients in the clinic. The strategy includes quality control steps to establish cell lines for further hiPSC-CM differentiation. The hiPSC-CMs are then matured and cultured as 2D monolayers or 3D tissue, followed by phenotypic assays that describe the mutant cell line at a transcriptomic-proteomic and functional level (Fig. 1). Fundamentally, the techniques described will streamline characterization of inherited arrhythmias in patient-specific hiPSC-CMs from bench-to-bedside.

2 Establishing Patient-Derived hiPSC Lines

2.1 hiPSC Reprogramming

Retroviral transduction of four transcription factors (Oct3/4, Klf4, Sox2, and c-Myc), now colloquially coined the "Yamanaka factors," was shown to reprogram mouse embryonic and human adult fibroblasts into an embryonic stem cell-like state (Takahashi and Yamanaka 2006). The group then reprogrammed human adult dermal fibroblasts from skin punch biopsies with the Yamanaka factors into hiPSCs (Takahashi et al. 2007). Seki, Yuasa, and Fukuda (2012) developed a protocol to generate hiPSCs from terminally differentiated T cells in a small blood sample. Together with a less invasive approach to obtain patient cells, they also delivered the Yamanaka factors into the cells using a mutant Sendai virus that, unlike integrative retroviruses, could replicate within the cytoplasm and subsequently is destroyed (Seki et al. 2012). Additionally, non-viral and vector-free delivery of these transcription factors is recommended to mitigate unwanted tumorigenesis (Malik and Rao 2013; Narsinh et al. 2011).

2.2 hiPSC Quality Control and Genotyping

Careful consideration must be given to the viability, fidelity, and stability of patient (and any commercially obtained) hiPSC lines cultivated in vitro. The hiPSCs are taken through quality control (QC) processes after being generated from either peripheral blood mononuclear cells (PBMCs) or dermal fibroblasts. QC steps can include, but are not limited to:

- Genetic stability – Assessment of genetic stability by karyotyping to ensure 23 pairs of chromosomes and/or quantitative PCR (qPCR) detection of common karyotypic abnormalities (e.g., hPSC Genetic Analysis Kit, STEMCELL Technologies, Vancouver, Canada).
- Sterility – Routine mycoplasma detection methods are PCR-based mainly due to easy sample preparation for rapid mycoplasma screening (e.g., Mycoplasma PCR Detection Kit, Applied Biological Materials Inc., Richmond, Canada). Bacterial, fungal, and viral testing are also recommended.

- Pluripotency – Validation of hiPSCs' differentiation into ecto-, meso-, and endodermal germ layers (e.g., STEMdiff Trilineage Differentiation Kit, STEM Technologies, Vancouver, Canada). Alternatively, an in-house qPCR or droplet digital PCR (ddPCR) assay can screen for relative (to housekeeping genes) or absolute expression, respectively, of pluripotency markers, such as Nanog (intracellular), Oct4 (intracellular), and TRA-1 (extracellular) transcripts.

Genotypic analysis of the patient cell line should be conducted, first by screening the genome against a panel designed for a specific disease as deemed by clinicians and researchers. The genome may then be studied further either by whole-exome sequencing or whole-genome sequencing, in combination with data from GWAS (Behr et al. 2013; Milan et al. 2010; Tucker and Ellinor 2014). The patient may have single nucleotide polymorphisms (SNPs) that are above a given P-value significance threshold identified in GWAS that may be associated with a particular disease phenotype; the first example of this SNP identification in AF was rs2200733 in chromosome 4q25 associated with AF (OR $\geq$ 1.6) (Gudbjartsson et al. 2007). In addition, sporadic or familial studies may require exon sequencing for genes encoding specific sarcomeric elements or ion channels to find amino acid changes that may affect protein interactions or structure (Dewar et al. 2017; Ellinor et al. 2010; Roston et al. 2018; Shafaattalab et al. 2019a). At this stage, the hiPSCs can be deposited in a biobank for other researchers worldwide to access for study, particularly if there are specific genetic variants, diagnoses, or arrhythmic patient phenotypes of interest.

2.3 Genome Editing Applications

Genome editing is a powerful tool for modeling arrhythmias and disease in hiPSC-CMs (Anzalone et al. 2019; Doudna and Charpentier 2014; Rees et al. 2019). With CRISPR/Cas9 genome editing, a homology-directed repair can facilitate precise base pair changes, as well as large insertions, using an exogenous DNA template (Ran et al. 2013). This technique is relatively straightforward, however; the editing efficiency in hiPSCs and other cell types remains inconveniently low (<3%). Some groups have taken the CRISPR/Cas9 system to new heights with modifications that increase editing efficiencies several fold and eliminate the necessity for double-strand breaks prone to insertions and deletions in non-homologous end-joining repair. Base and prime editing are two CRISPR-based genome editing techniques developed in the Liu lab at the Broad Institute of MIT and Harvard University; base editing utilizes a cytidine or adenine deaminase complexed to a Cas9 nickase to convert cytosine bases to thymine via a uracil intermediate or adenine bases to guanine via an inosine intermediate (Gaudelli et al. 2017; Komor et al. 2016). Prime editing has been making headlines due to the sophisticated design of the system and drastically improved editing efficiencies compared to traditional CRISPR/Cas9 (Anzalone et al. 2019). The protocol involves a Cas9 nickase, but this time fused to a modified Moloney leukemia virus (M-MLV) reverse

transcriptase. A prime editing guide RNA (pegRNA) mediates both specific annealing to a target region of interest on the DNA strand, as well as a template sequence that the reverse transcriptase uses to add nucleotides along with the nick (Anzalone et al. 2019). The system is precise and can mediate all 12 point mutations, and smaller insertions and substitutions up to 40 bp long (Anzalone et al. 2019, 2020). Prime editing efficiencies rely heavily on the initial design; notably, the Olson lab at the University of Texas Southwestern pioneered one of the first studies to use prime editing in hiPSCs. They could achieve impressive 20–54% editing efficiencies for a two-nucleotide exon correction in the dystrophin gene (Chemello et al. 2021).

Using the genome editing tools described, there is the freedom to make almost any desired base pair changes and targeted insertions. Importantly, isogenic controls of a patient cell line can be established by correcting suspect genetic variants (Hoekstra et al. 2012). One can reliably compare patient hiPSC-CM assay results with a control line matching their genetic signature by establishing a "corrected" version of their genome. To further investigate a potentially arrhythmogenic variant across a diverse range of genomes, variants of interest can be introduced into "wild-type" cell lines. These hiPSC lines can be procured from phenotypically healthy individuals, as well as commercial sources. Fluorescent tags like genetically encoded voltage indicators (GEVIs) and genetically encoded calcium indicators (GECIs) can also be used during live-cell imaging to track the electrical changes across the cell membrane to indicate action potential activity and morphology (Broyles et al. 2018; Shinnawi et al. 2015). The generation of GFP-sarcomere reporter lines can allow for direct contractility analysis using fast Fourier Transforms (FFTs) or similar tracking applications for further characterization of arrhythmias and associated cardiomyopathies (Sharma et al. 2018; Toepfer et al. 2019). Applications using these fluorescent tags, as well as establishing mutant and isogenic patient-specific hiPSC lines, are crucial in dissecting inherited arrhythmias and will be discussed later in this chapter.

3 hiPSC-CM Differentiation, Purification, and Maturation

3.1 Differentiation and Definition of Homogenous Cardiomyocyte Subpopulations

Protocols for hiPSC-CMs differentiation generally involve temporal modulation of the canonical Wnt/β-catenin signaling pathway by the addition of small molecules in defined culture media (Burridge et al. 2014; Lian et al. 2013) (Fig. 2). The previously described protocols typically result in 60–85% ventricular-like, 5–30% atrial-like, and 3–15% nodal-like hiPSC-CMs subpopulations with the remainder of the population containing fibroblasts or other non-cardiac cells (Devalla et al. 2015; Hoekstra et al. 2012; Itzhaki et al. 2011; Laksman et al. 2017; Protze et al. 2017). Using more specific cardiac cell types may be useful depending on the arrhythmia being investigated. For example, the retinoic acid signalling pathway has been shown as a regulator of both atrial and nodal lineages. Thus an increase in the percent population of atrial cells and nodal cells can be achieved with the timed addition of retinoic acid

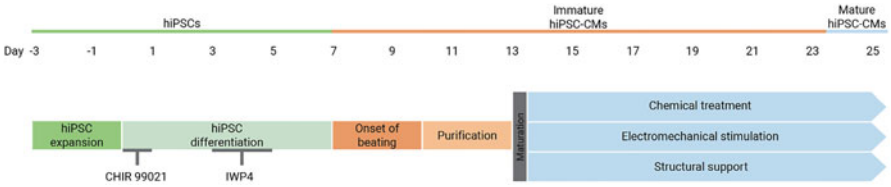

Fig. 2 Directed differentiation of hiPSCs into immature, fetal-like hiPSC-CMs. Following population purification, chemical, electromechanical, and/or structural treatments are used to induce maturation to produce hiPSC-CMs with more adult-like phenotypes

during the differentiation process (Gunawan et al. 2021; Lian et al. 2013; Protze et al. 2017). Other strategies to generate chamber-specific cells have also used directed differentiation protocols combined with electrical conditioning to create electrophysiologically distinct atrial and ventricular tissue (Zhao et al. 2019).

The cardiomyocyte population can be further purified from non-hiPSC-CM cells, which may be vital to avoid confounding data from these cells in the phenotypic assays described later in the chapter (Fig. 2). Enrichment of the cardiomyocyte cell population can be achieved through cardiomyocyte-specific antibody selection combined with a magnetic column (e.g., PSC-Derived Cardiomyocyte Isolation Kit, Miltenyi Biotec, Auburn, CA, USA). Alternatively, metabolic selection, which employs a change from regular maintenance media to glucose-depleted and supplemented with sodium L-lactate, can be used to eliminate non-cardiomyocyte cell types (Feyen et al. 2020). The principle behind this strategy is that cardiomyocytes, unlike most other cell types, preferentially use free fatty acids (FFAs) as a substrate for energy consumption but can use other substrates as well. In the absence of glucose, CMs are still able to efficiently produce energy from lactate and survive (Tohyama et al. 2013). However, non-cardiomyocyte viability is low with a multi-day treatment of this metabolic selection media, and thus, they are eliminated from the culture. It is important to note that the co-culture of cardiac fibroblasts or endothelial cells is a promising strategy for structural and electrophysiological hiPSC-CM maturation (Beauchamp et al. 2020; Kim et al. 2010b). Thus, these non-cardiomyocytes may need to be cultured separately and mixed with a specific hiPSC-CM population in a defined quantity. With near 100% hiPSC-CM subtype specification, modeling arrhythmias and therapeutic drug screening are more refined for further translation to in vivo patient cell phenotypes.

3.2 Maturation

Maturation of the hiPSC-CMs remains one of the most significant barriers to their application in research and clinical therapeutics. While they are still able to recapitulate many arrhythmogenic phenotypes observed in patients and other models (e.g., murine models, biochemical studies), hiPSC-CMs produced under standard differentiation protocols retain many of the structural and functional qualities of a fetal

cardiomyocyte. Current research is focused on tackling the challenge of simulating the cardiomyocyte maturation process in vitro (Karbassi et al. 2020; Lundy et al. 2013; Marchianò et al. 2019; Piccini et al. 2015; Sun and Nunes 2017), though complicated and not fully achieved yet. Many strategies for hiPSC-CM maturation are outlined in the literature and encompass guided electrical, chemical, and metabolic treatments (Feyen et al. 2020; Garbern et al. 2020; Ronaldson-Bouchard et al. 2018; Sun and Nunes 2017). In addition, the growth of hiPSC-CMs as 3D tissue, such as organoids, spheroids, or microtissue mounted on structural scaffolding, has helped to enhance some phenotypes that are missing in immature 2D tissue, including the development of T-tubules (TT) for excitation-contraction coupling (Kim et al. 2010a; Parikh et al. 2017; Ronaldson-Bouchard et al. 2018; Sun and Nunes 2017). Integration of these approaches, which involve mechanochemical and metabolic cues, could help activate multiple, cascading molecular signalling pathways to enact developmental changes in the cells ranging from transcriptional and protein expression to tissue and organ morphology and function (Fig. 2).

4 Phenotypic Assays

4.1 Transcriptomic and Proteomic Analysis

Most studies of this nature employ transcriptional analyses such as real-time quantitative PCR (RT-qPCR) to give insight into the average expression profile relative to housekeeping genes of the hiPSCs or hiPSC-CMs. However, newer techniques like ddPCR, Nanostring, and bulk- and single-cell RNA-seq can capture a high-resolution, high-throughput snapshot of transcript heterogeneity within a cell population (Geiss et al. 2008; Paik et al. 2020; Taylor et al. 2017). Transcriptomics can be applied to quantifying the expression of markers for pluripotency, cell subpopulations post-differentiation, and markedly, cardiac maturation. In the context of modeling arrhythmias, transcript expression of genes associated with intracellular Ca^{2+} handling, ion channel expression, contractile function, and gap junction coupling between hiPSC-CMs can be assessed (Guo et al. 2019; Kamdar et al. 2020). While transcriptional analysis is useful, RNA expression levels often do not reflect protein concentrations due to differential rates of transcription and translation or protein trafficking within the cell. Different stressors and physiological demands also affect RNA and protein expression levels; thus, protein expression analysis should be integrated when phenotyping arrhythmias in hiPSC-CMs. Protein characterization is traditionally conducted using SDS-PAGE and western blotting, however; mass spectrometry (MS)-based proteomics provides a more detailed, more quantitative, non-biased, and meticulous account of protein up or downregulation, isoform distinction, and posttranslational modifications (PTMs). Bottom-up MS proteomics is a high-throughput method of quantifying global protein regulation by digestion of proteins into peptides. Alternatively, a top-down approach of MS-based proteomics is recommended by Cai et al. (2019) in hiPSC-CMs for increased specificity of protein targeting. Compared to bottom-up proteomics, the proteins are kept intact for

full sequence coverage, allowing for more refined isoform and PTM identification (Cai et al. 2019). Top-down proteomics has been used to assess hiPSC-CM maturation markers (Cai et al. 2019) and characterize proteomic changes in explanted septal tissue from HCM patients, including PTMs such as phosphorylation of the troponin complex subunits (Tucholski et al. 2020). Together with transcriptomic analysis in hiPSC-CMs, signaling pathways and molecular changes in inherited arrhythmias can be linked to the clinical manifestation of the disease.

4.2 Metabolic Considerations

One of the critical markers of cardiomyocyte maturation is the metabolic switch from glycolysis to fatty acid oxidation, occurring during fetal development (Batho et al. 2020; Hu et al. 2018; Lin et al. 2017; Nakano et al. 2017). This transition is coupled to producing many sarcomere-arrayed mitochondria for high oxidative phosphorylation capacity and energy yield from free fatty acids (Piquereau and Ventura-Clapier 2018). Media containing FFAs and other chemical substrates can be applied to hiPSC-CMs to facilitate a switch in their metabolism, which is largely glycolytic (Feyen et al. 2020). There is also a hypothesis that sarcomeric HCM mutations contribute to poor ATP use and thus energy depletion, which may be further explored (Ashrafian et al. 2003). Changes in hiPSC-CMs' energetics can be assessed using a Seahorse Analyzer assay (Agilent Technologies, Santa Clara, CA, USA), yielding critical information about the metabolic state of the hiPSC-CMs (Feyen et al. 2020). In this assay, a calibrated sensor near the cell surface detects changes in both the pH and the O_2 concentration in the surrounding media. These analyses provide a measure of cellular processes such as the oxygen consumption rate (OCR; a measure of cellular and mitochondrial respiration over time reported in picomole/minute), extracellular acidification rate (ECAR; a measure of proton extrusion into the extracellular medium over time reported in mili-pH/min), and proton efflux rate (PER; a measure of extracellular acidification accounting for media buffering capacity and plate geometry over time, reported in picomole/minute). These data give insight into the metabolic state of the cells and can be correlated with expression and morphological changes in mitochondrial size, quantity, development, and organization.

4.3 hiPSC-CM Morphology

Morphological analysis is a powerful aspect of hiPSC-CM phenotyping to identify biological perturbations, such as gap junction uncoupling and loss of TTs, which may contribute to electrical disarray and arrhythmias. Transmission electron microscopy (TEM) offers the highest resolution view of cellular ultrastructure as detailed as the dyadic coupling of L-type calcium channels (LTCC) and ryanodine receptors (RyRs) at the sarcoplasmic reticulum (SR). Both cardiomyocyte development and dysfunction can often be linked to pathological changes at the ultrastructural level.

Despite being a low-throughput technique, TEM data offers a wealth of information about the state of the cell. For a global assessment of cell and tissue morphology, a method from the Carpenter lab may be used. Cell painting involves multiplexing several fluorescent dyes and/or antibodies (such as those used in immunocytochemistry) to produce a high-throughput morphology screening tool (Bray et al. 2016). Visual readouts from each detection channel can allow extraction and analysis of 1500+ features, including shape, texture, and spatial relationships between stained (Bray et al. 2016). Morphological profiling with cell painting can also be used to assess the efficacy of hiPSC-CMs development and maturation, as well as the acute or chronic effects of certain drug treatments on labeled cellular structures. Hints as to the mechanism of action of an arrhythmia may be revealed in an unbiased manner based on observed morphological changes.

4.4 Contractility and Force Generation

The ability of cardiomyocytes to generate force is dependent on several factors, including the structural alignment of the sarcomeres, cytosolic Ca^{2+} handling, temperature, and drug effects. The physiological mechanisms of contractility are reasonably well understood (Chapman 1983; Kobayashi and Solaro 2005). However, disease mechanisms for contractility-related diseases, such as hypertrophic cardiomyopathy, are still not well understood. Although there are several theories as to the mechanisms of dysfunction, more research is necessary to determine how and why Ca^{2+} mishandling results in structural remodeling and arrhythmogenesis. Various aspects of contractile properties can be measured through several techniques encompassing FFT analysis, edge detection, and impedance-based microarrays. An important, high-throughput MATLAB software called SarcTrack can individually track fluorescently labeled sarcomeres in hiPSC-CMs and assess sarcomere content, beat rate, and calculate the rate of contraction and relaxation (Toepfer et al. 2019). In addition, atomic force microscopy (AFM), can also be used to determine the stiffness and force of contraction of the contractile unit (Borin et al. 2018; Chang et al. 2013). Evaluation of these functional properties of the hiPSC-CMs would provide critical information about disease states and provide a means to test how the cells respond to pharmacological agents that carry cardiotoxic risks.

4.5 Electrophysiological Measurements and Arrhythmia Assessment

Cardiomyocyte function is greatly dependent on the electrophysiological properties of cells at the single-cell level or as part of a functional syncytium. Three main techniques can be used to assess electrophysiology. Microelectrode array (MEA) systems, like the Maestro Pro multi-well MEA and impedance system (Axion Biosystems, Atlanta, GA, USA), serves to assess electrical activity and contractility in response to perturbations like drug additions or pH changes. Electrodes embedded

in each well provide a method for applying electrical stimulation to the cells and recording field potentials across the tissue layer. Changes in hiPSC-CM electrophysiology can be continuously observed over days or weeks due to the method's non-invasive approach.

Optical mapping is an imaging technique that uses dyes or genetically encoded reporters (e.g., GECIs) for the determination of membrane voltage (e.g., RH-237, Fluovolt) and Ca^{2+} (e.g., Rhod2-AM, Fluo-4) (Lin et al. 2015; Shafaattalab et al. 2019b). This technique can provide high spatio-temporal resolution across a tissue that can then be used to access action potential morphology, propagation across tissue, and Ca^{2+} handling in response to arrhythmogenic triggers such as beta-adrenergic stimulation, increase packing rate, or arrhythmogenic drugs. Light-gated ion channels can be used to either stimulate or quiesce (e.g., BLINK 2) cell movement during imaging. The latter can be used to mitigate movement artifacts during live-cell imaging, without using drugs like blebbistatin, a commonly used myosin II inhibitor, or para-aminoblebbistatin, a photostable and non-fluorescent derivative of blebbistatin (Alberio et al. 2018).

Patch clamping, in many respects, is the "gold standard" of electrophysiological analyses and is a technique that allows one to measure either ion currents (voltage clamp) or membrane potential (current clamp) in single living cells. This technique is the only one of the three mentioned in this chapter that can be used to measure the absolute membrane potential (in mV) or ionic current densities (mA/pF). It is also the only means of examining the impact of genetic variants on ion channel biophysics. Patch clamping allows for the most accurate evaluation of the relative contribution of various ion channels to the cardiac action potential. However, there are disadvantages as well. First, to voltage clamp a cell, it is necessary to have a space clamp. This requirement is not possible in a functional syncytium as the cell-to-cell coupling allows the spread of electrotonic currents between cells. Thus, enzymatic (e.g., collagenase) cell dissociation is needed, which in itself can change cellular properties. Furthermore, arrhythmias can only be genuinely studied at a tissue (i.e., multicellular) level of organization. Thus, there are limitations to the conclusions one can make from examining a single cell. Secondly, this approach is the most technically challenging compared to others described here, both in data acquisition and data analysis. Thirdly, patch clamping is very labor intensive; the number of cells that can be examined is very limited and, therefore, prone to sampling errors. The second and third limitations may be mitigated somewhat by automated patch clamping instruments (e.g., Nanion PatchLiner, Sophion QPatch II), but they tend to be expensive.

In sum, all three of these techniques can be used to study cardiac electrophysiology, and each provides a different set of information that can be applied based on the requirements of the research question.

5 hiPSC-CMs for Proarrhythmic Drug Assessment

Electrical phenotypes may be unique on a case-by-case basis, and consequently, patients require extensive assessment and diagnosis that can only be enhanced by personalized medicine. Antiarrhythmic drug efficacy can be low and sometimes actually proarrhythmic. Nearly 90% of pharmacological treatments that have shown promise in research settings exhibit poor outcomes in Phase III clinical trials (Colatsky et al. 2016). Additionally, non-cardiac-related drugs have often been pulled after trial testing due to unexpectedly causing arrhythmias in test subjects. This discrepancy is often due to key physiological differences between the animal models used in the research setting compared to humans resulting in poor clinical translation. Thus, an initiative called the Comprehensive in vitro Proarrhythmia Assay (CiPA) was created to screen proarrhythmic risk of pre-clinical drugs (Colatsky et al. 2016). Because clinical trials are expensive, it is crucial that potential pharmacological interventions be tested for efficacy and cardiotoxicity in vitro. Using hiPSC-CMs, multiple drugs can be screened in parallel at no risk to the health of the patient. Thus, hiPSC-CMs present an attractive model for drug testing for pro-arrhythmic risk as they can model a given patient's genotype and phenotype.

6 Conclusion

Employing a systems biology approach allows investigators to corroborate electrical dysfunction in patients across many interdependent disciplines. Increased prevalence of hiPSCs in preclinical screening should provide significant insight to our knowledge of inherited arrhythmias that can be leveraged toward developing new therapeutic strategies. Furthermore, the capacity to model patient-specific genetic backgrounds allows one to observe patient-specific responses to different treatment approaches, thereby identifying reproducible pathophysiological characteristics as suitable drug targets for novel therapeutic interventions. Many studies to date have used hiPSC-CMs along a similar pipeline as described in this chapter to study arrhythmias, such as long QT syndrome (LQTS) (Moretti et al. 2010; Cagavi et al. 2018), hypertrophic cardiomyopathy (HCM) (Lan et al. 2013), dilated cardiomyopathy (DCM) (Sun et al. 2012), Brugada syndrome (Nijak et al. 2021), SCDs (Shafaattalab et al. 2019a), atrial fibrillation (AF) (Ahlberg et al. 2018; Benzoni et al. 2020; Laksman et al. 2017), and arrhythmogenic RV dysplasia (Khudiakov et al. 2017). In addition, the use of hiPSC-CMs in combination with novel biomaterials and tissue engineering technologies to enhance structural and functional development will enable a more comprehensive analysis of arrhythmic phenotypes and drug-induced functional changes in vitro. The extensive work to date highlights the array of technologies available to improve hiPSC-CMs development and maturation.

The focus must now be shifted toward integrating these technologies to produce physiologically and functionally accurate representations of adult myocardial tissue. However, adopting hiPSC-CMs tissue engineering strategies for improved disease

modeling will require the development of uniform procedures capable of addressing issues such as cardiomyocyte maturation and effective recapitulation of disease states. The various applications described are directed toward a common goal of bench-to-bedside characterization of arrhythmias in patient-specific hiPSC-CMs. High-throughput, disease-specific assays screened against focused libraries may allow for the discovery of candidate therapeutic targets through the interrogation of the entire transcriptome and proteome, thus resulting in a powerful paradigm shift in drug discovery and therapeutic strategies.

7 Cross-References

- ▶ Advances, Opportunities and Challenges in Stem Cell-based Therapy
- ▶ Bioengineering Technique Progress of Direct Cardiac Reprogramming a New Perspective from Microbubbles and UTMD
- ▶ Current State of Stem Cell Therapy for Heart Diseases
- ▶ Induced Pluripotent Stem Cells Progress Towards Clinical Translation from Bench to Bedside
- ▶ Molecular Signature of Stem Cells Undergoing Cardiomyogenic Differentiation
- ▶ Stem Cell Applications in Cardiac Tissue Regeneration
- ▶ Therapeutic Uses of Stem Cells for Heart Failure
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